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Impact of culture medium on the expansion of T cells for immunotherapy

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Background aims

Encouraging evidence of clinical benefits from cancer immunotherapy is beginning to accumulate in several clinical trials. Cancer immunotherapy is based on two main methods, active vaccination and cell-transfer therapy. The ex vivo *expansion of T cells is required to monitor vaccine-induced antigen-specific T cells or prepare large numbers of reactive lymphocytes for adoptive transfer.*

Methods

We examined the influence of culture medium on T-cell growth, cytotoxicity and phenotype after activation using immobilized anti-CD3 monoclonal antibody or Zoledronate stimulation. Peripheral blood mononuclear cells (PBMC) were cultured in RPMI, AIM-V or OpTmizer with or without autologous serum.

Results

When supplemented with sufficient serum, RPMI was a good culture medium for T-cell expansion following anti-CD3 stimulation. Addition of autologous serum to AIM-V or OpTmizer increased the numbers of cells obtained to a similar extent, but their phenotype and function were quite different. Activated T cells cultured with OpTmizer mediated greater cytotoxicity than any other culture. Regardless of the media used, the main population expanded after CD3 stimulation was $CD3^+$ $CD8^+$. While more $CD3^+$ $CD4^+$ T cells were induced in RPMI and AIM-V, more $CD3^-$ $CD56^+$ cells and $CD3^+$ $CD56^+$ T cells were induced in OpTmizer. When cells were stimulated by Zoledronate for 14 days, approximately 7.2 times and 11.5 times more γδ T cells were obtained in OpTmizer than AIM-V or RPMI, respectively.

Conclusions

Successful immunotherapy depends on the selection of appropriate culture media to support efficient expansion of the type of T cell desired.

Keywords

AIM-V, anti-CD3, culture media, γδ T cell, immunotherapy, OpTmizer, RPMI, T-cell expansion, Zoledronate.

Introduction

The potential of cancer immunotherapy is promising. Better understanding of tumor-specific immune responses, identifying tumor-associated antigens (Ag) and manipulating the immunoregulatory environment of the tumor is likely to increase further the efficacy of cancer immunotherapy [1]. T-cell based cancer immunotherapy is of two types, active cancer vaccination and adoptive cell transfer [2].

To develop cancer vaccines, immune monitoring to determine the efficacy of vaccine-induced Ag-specific T-cell responses is crucial [3]. Ag-specific T cells can be detected by staining the T-cell receptor with major histocompatibility complex (MHC) peptide multimers or measuring cytokine production in response to Ag, such as by ELISPOT and intracellular cytokine flow cytometry [4]. However, the frequencies of tumor-specific T cells are usually too low to detect directly *ex vivo*. Several rounds of *in vitro* T-cell expansion are commonly required to visualize responses. For this purpose, culture conditions supporting specific responses but low non-specific background reactivity are essential.

Adoptive cell transfer has shown remarkable clinical results in patients with metastatic melanoma, with approximately 50% of patients experiencing an objective

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response [5]. A crucial step for this strategy is the rapid, large-scale *ex vivo* expansion of tumor-reactive T cells in the presence of interleukin-2 (IL-2), anti-CD3 antibody (Ab) and irradiated peripheral blood mononuclear cells (PBMC) as feeder cells [6].

Recently, we developed a protocol for the large-scale *ex vivo* expansion of γδ T cells for use in adoptive transfer therapy [7]. During the establishment of this approach, we noted large differences between results using different culture media to expand T cells. Therefore, we systematically compared three defined cell culture media, RPMI-1640 (RPMI), AIM-V and OpTmizer T Cell Expansion SFM (OpTmizer), in terms of their support of the proliferation and function of T cells activated by immobilized anti-CD3 Ab or Zoledronate.

Methods

Isolation of PBMC and T-cell culture

Culture media were all from Invitrogen (Grand Island, NY, USA). RPMI was supplemented with 2 mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate, 100 μg/mL penicillin and 100 U/mL streptomycin. AIM-V was used as supplied. OpTmizer was supplemented with 26 mL/L OpTmizer T Cell Expansion Supplement and 2 mM L-glutamine, according to the manufacturer's instructions.

Informed consent was obtained from six healthy donors prior to blood collection. All procedures were approved by the Ethical Committee of the Graduate School of Medicine, University of Tokyo (Tokyo, Japan). Whole blood (7.5 mL) was collected in BD Vacutainer blood collection tubes with sodium heparin (BD, Franklin Lakes, NJ, USA) and directly centrifuged to isolate PBMC. PBMC were suspended at a final concentration of 1.0×10^5 cells/mL in the indicated medium and cultured in 24-well plates. Cells were activated with 3.3 μg/mL plate-bound anti-CD3 monoclonal antibody (MAb) (muromonab-CD3; Janssen Pharmaceutical K.K., Tokyo, Japan). Cultures were maintained at $0.5–2\times10^6$ cells/mL and supplemented with 175 IU/mL human recombinant (r)IL-2 (Proleukin™; Chiron, Amsterdam, the Netherlands) every 2–3 days. To stimulate and expand γδ T cells, PBMC were also stimulated with 5 μM Zoledronate (Novartis, Basel, Switzerland) in the indicated culture media, as described previously [7]. Medium containing IL-2 (1000 IU/mL) was added every 2–3 days. T-cell cultures were monitored using an inverted microscope IX71 (Olympus, Tokyo, Japan). The cultures were transferred into new flasks as necessitated by the degree of cell growth. Cultured cells were analyzed at the indicated time-points. To prepare serum, whole blood (10 mL) in 15-mL conical tubes was stored for 1 h at room temperature. It was then centrifuged at 3000 r.p.m. for 10 min and the supernatant transferred into a new tube. Heat inactivation was performed at 56°C for 1 h.

Flow cytometry

The following MAb were used for phenotypic analysis: fluorescein isothiocyanate (FITC)-labeled anti-CD3 and TCRVγ9; phycoerythrin (PE)-labeled anti-CD56 and mouse IgG1; phycoerythrin-Cy5 (PC5)-labeled anti-CD3, CD8 and mouse IgG1; and phycoerythrin-Texas Red-X (ECD)-labeled anti-CD4, CD45 and mouse IgG1. These were all purchased from Beckman Coulter (Immunotech, Marseille, France). The PBMC absolute cell count was determined by the addition of Flow-Count Fluorospheres (Beckman Coulter) and cell viability was determined by staining with 20 μg/mL 7-aminoactinomycin D (7-AAD) (Sigma, St Louis, MO, USA). The cells were stained with Ab and analyzed using a Cytomics FC 500 (Beckman Coulter, Fullerton, CA, USA). The data were processed using CXP Analysis 2.0 software (Beckman Coulter).

Table I. Culture media used in this study.

	RPMI-1640	AIM-V	OpTmizer T Cell Expansion SFM
Application	Mammalian cell	Human lymphocyte	Human T lymphocyte
Serum	Required	Free	Free
Supplement	None	Human albumin	Human albumin, NAC
Grade	Research	Research/therapeutics[a]	Research

[a]For human *ex vivo* tissue and cell culture-processing applications.